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Effect of Aqueous Extract of *Vernonia amygdalina* on Biochemical Indices of Prostate Functions in Hormonal Induced Enlarged Prostate in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors UMN and EMU designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MMA managed the analyses of the study. Authors UMN and MMA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Benign prostate hyperplasia (BPH) is a common urological disorder in elderly men. We investigated the effect of aqueous extract of *Vernonia amygdalina* (VA) on BPH.
Methods: BPH was induced in male rats weighing 250-350 g. Testosterone propionate (T) and estradiol valerate (E_2) were used for the induction at a dose of 400 µG T and 80 µG E_2 respectively. This was administered to the rats for three weeks subcutaneously in the inguinal region. A total of 30 rats were divided into five groups. One group was used as a control and the other groups received subcutaneous injections of the hormones for 3 weeks to induce BPH.

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Groups 1 and 2 were treated with different doses of VA extracts and group 3 received finasteride, all by gavages for forty-five days, while group 4 was left untreated, group 5 served as normal control. After forty-five days of treatment with VA extract, the rats were anaesthetised by short contact with trichloromethane vapour. Blood was collected by cardiac puncture and the sera cautiously centrifuged and used for the determination of different biochemical indices. The prostate was harvested and weighed. The harvested prostate were processed for paraffin embedding and stained with H and E.

Results: Treatment with the extract and finasteride resulted to significant ($P < 0.05$) decrease in prostate-specific antigen (PSA), estradiol and prolactin, testosterone and protein content of the prostate when compared to BPH control. Prostate weight was significantly ($P < 0.05$) reduced in treated groups compared to BPH control. This was supported by the histological examination.

Conclusion: Therefore, *Vernonia amygdalina* was effective in reducing PSA, prolactin, testosterone, estradiol and prostate weight induced BPH in a rat model, and may be useful for the clinical treatment of patients with BPH.

Keywords: Estradiol; PSA; prostate; testosterone and Vernonia amygdalina.

1. INTRODUCTION

Benign prostatic hyperplasia (BPH) is the most common benign urologic disorder in older men. Severe symptoms related to BPH can cause the quality of life to deteriorate, and treatment for BPH has serious economic implications [1]. An estimated 75% of men older than 50 years have symptoms arising from BPH, and 20% to 30% of men reaching 80 years require surgical intervention to manage BPH [2,3].

Although the pathogenesis of BPH is not fully understood, the relationship between the concentrations of androgens (male sex hormones) and BPH in aging men has been established [4,5]. In line with this, there have been two known etiologic factors involved in the pathogenesis of BPH: ageing and testicular androgens [6]. In addition, recent novel findings have highlighted the role of metabolic syndrome, diabetes, obesity, cigarette smoking, hyperlipidemia, insulin resistance, and inflammation [7,8].

Vernonia amygdalina is commonly called Bitter leaf in English language, Onugbu in Igbo language; it is called Etidot in Efik, Ijaw and Ibibio, Ewuro in Yoruba language, Oriwo in Edo and Chusa-doki in Hausa [9]. In many parts of Nigeria, the plant has been domesticated and used in the treatment of various infection and diseases. The leaves are extremely bitter because of its composition [10], which gives the plant its therapeutic capacity [11,12]. *Vernonia amygdalina* (VA) is reported to have a broad spectrum of medicinal relevance [13,14,15,16]. Report from [17] revealed that compounds from the crude extract of *V. amygdalina* demonstrated the ability to inhibit proliferation of cell in prostate cancer cells. It has been reported that *V. amygdalina* extracts can enhance the sensitive of cancer cells to chemotherapy [18]. *V. amygdalina* has been recognized to be implicated in the inhibition of the growth of cancerous cells [19, 20]. Several investigations have indicated that *V. amygdalina* extracts have the capacity to boost the immune response in rats [18]. This study investigated the usefulness of the leaf extract of *Vernonia amygdalina* in the management of the experimentally hormone-induced BPH in Wistar rats. The results will contribute to the search for locally available phytotherapeutic agents that can help in managing this debilitating disease especially in among the poor ones.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Vernonia amygdalina* was harvested from a garden in Okuku in Yala Local Government of Cross River State, South-South, Nigeria. The plant was identified at the herbarium unit of the Department of Biological Sciences, University of Calabar. Their fresh leaves were washed with clean water and dried under the shade for six days. Their dried leaves were milled using pestle and mortar to get a powder that was used for extraction.

2.1.1 Preparation of extract

The powdered sample of *Vernonia amygdalina* 100 g was soaked into 100 ml of distilled water, this was filtered after 48 hours and filtrate was concentrated in water bath. The solutions were diluted with corn oil, to produce a solution 100

mg/ml. The administration of extract was totally by gavage. Proper concentrations were administered by the use of oropharyngeal canula and calibrated hypodermic syringe.

2.2 Hormones

Testosterone propionate Brand name: Ricostrone; a product of Greenfield pharma, Jiangsu Co Ltd., China. Estradiol valerate (by Medipharm Ltd., 108-Kotlakhpat industrial Est; Lahore, India. Testosterone propionate (T) and estradiol valerate E_2 (puregynon depot) were used for the induction of prostate enlargement at a dose of 400 µG T and 80 µG E_2 [21]. This was administered to the rats for three weeks subcutaneously in the inguinal region after which a few rats were sacrificed and inspected for gross examination of prostate enlargement. All Chemicals used in this study were of analytical grade and were obtained from reputable companies.

2.3 Animals

A total of thirty (30) Wistar rats weighing between 250-350 g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The rats were used for the experiment. The rats were acclimatized for two weeks before the experiment commences. The rats were exposed to approximately 12-hour light/dark cycles under humid tropical conditions, given tap water and feed *ad libitum*, and were housed in standard plastic cages (five per cage) throughout the 45-day duration of the study. The animal room was well be ventilated with a temperature range of 27-29°C. The Institutional Animal Ethics Committee approved the study before the experiment and certified all experimental protocols.

2.3.1 Induction of BPH

BPH was induced by exogenous administration of testosterone and estradiol in staggered doses (three times a week respectively) for three weeks according to [21] with modification by [22].

2.3.2 Animal grouping and treatment

The animals were divided into five (5) groups each comprised of six (6) male rats. Four groups were induced with BPH which were grouped as group 1 to group 4). Groups 1 and 2 received 50 and 100mg kg^{-1} body weight (bw) of *Vernonia amygdalina* extract; group 3 received finasteride (orthodox drug) at 0.1 mg kg^{-1}; all by gavages for forty five days, group 4 was left untreated for forty five days before sacrifice to assess possible reversal of the exogenous induction and group 5 served as normal control. The animals were weighed prior to the commencement of the experiment and subsequently every week till the end of the experiment. The fluid and water intake was taken daily till the end of the experiment.

2.4 Determinations of Biochemical Parameters

After 45 days, the rats were anaesthetized by a brief exposure to trichloromethane vapor and bled by cardiac puncture. Blood samples were collected and transferred into vacutainers without anticoagulant, and serum was separated by centrifugation at 2,500 rmp for 15 min using bench top centrifuge (MSE Minor, England). After centrifugation serum samples were collected using dry Pasteur pipette and stored in the in a freezer at -20℃ until use. All analyses were completed within 24 h of sample collection.

Each rat's carcass was promptly dissected and the prostates were carefully excised. Two prostates per group were randomly selected and their dorso-lateral lobes were dissected out and immediately processed for histology. The other three prostates pre group were freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance. Subsequently, they were homogenized in ice-cold normal saline and the homogenates were used for the determination of the protein content of the prostate.

2.4.1 Determination of PSA

Serum PSA was determined using TC-96+ Teco ELISA microplate reader manufactured by Teco Diagostic Laboratory, USA. The ELISA test is based on the principle of solid phase enzyme linked immunosorbent assay, where the antibody to be measured is incubated with specific antigen coupled to a solid phase [23]. PSA molecule was sandwiched between solid phase (rabbit anti-PSA antibody) and enzyme linked antibodies (monoclonal anti-PSA conjugated to Horse raddish peroxidase). After removing the unbound-labelled antibodies, tetramethyl benzidine (TMB) was added as substrate for the conjugated enzyme to digest resulting into colour complex that is proportional to the concentration of PSA in the serum [24].

2.4.2 Determination of serum prolactin, testosterone and estradiol concentrations

A solid phase enzyme immunoassay (EIA) quantitative method was employed for the determination of the concentration of each hormone in the serum. The prolactin protocol utilizes two antibodies directed against distinct antigenic determinants of the prolactin molecule as described by [25]. Rabbit anti-prolactin rabbit polyclonal antibody which had been enzyme-labeled with prolactin-horseradish peroxidase was used.

The testosterone protocol was based on the method of [26] and involves the competition of testosterone in serum and enzyme-labeled testosterone for binding with anti-testosterone antibody immobilized on the microwell surface (rabbit anti-testosterone antibody and testosterone-horseradish peroxidase (HRP) Conjugate). The estradiol protocol also utilizes the competitive binding principle as described by [27] where estradiol is sandwiched with rabbit anti-estradiol antibody and estradiol-biotin conjugated to avidin-horseradish peroxidase.

2.4.3 Determination of protein content of the prostate

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of colored complex. The protein content of the prostate was determined using the modified Biuret method of Feinstein [28]. Briefly, 3.9ml of deionized water and 4.0 ml of Biuret reagent were added to 0.1 ml of the aliquot and allowed for 30 minutes at room temperature to develop. A standard and blank were also prepared by adding 4.0 ml of Biuret reagent and 3.9 ml of deionized water to 0.1 ml of standard albumin and water respectively. Subsequently, the absorbance of the test and standard were read against the blank at 540 nm using a UV/VIS spectrophotometer.

2.5 Histological Studies

The prostate gland was washed in 0.9% physiological (normal) saline before it was fixed in 10% formal saline for 48 hours. It was later transferred into 70% alcohol, two changes for two hours each and to 90% alcohol, two changes for two hours each. This was then transferred to absolute alcohol of two changes each for two hours. The tissue was then removed to a mixture of equal volumes of alcohol and xylol, and then transferred to two changes of xylol for two hours each to produce clear tissue. The clear tissue was then transferred to molten paraffin wax of melting point 52°C. The wax was kept at this temperature in a thermostatically controlled bath with two changes of bath at one hour each. The tissue was later embedded in molten paraffin wax and allowed to solidify. The embedded block was trimmed and sections were cut from the block at 5 micron meter each. The tissue was the floated on water bath and mounted in clean albumenized slide. It was allowed to dry in an incubator for 24 hours at 37°C and was later stained with H and E (hematoxylin and eosin) and was mounted in Canada balsam. Microscopic examinations of the sections were then carried out under a light microscope.

2.6 Statistical Analysis

The experimental data were analysed for statistical significance, normal distribution of the data was evaluated prior by one-way analysis of variance and post hoc comparison using the SPSS version. All data were reported as mean ± SD and statistical significance was accepted at $P < 0.05$.

3. RESULTS

3.1 Weekly Body Weight

The BPH-control group exhibited a decline in body weight by 19% (270.4 g) when compared with normal control (without BPH, 322.2 g) and there was a declined appetite after three weeks of BPH induction. The 50 mg VA, 100 mg of VA and finasteride exhibited 1.45% (317.60), 0.19% (321.60) and 0.56% (320.40) decline in weight respectively when compared to the normal control, reaching the weight close to the normal control group (322.2 g). Finasteride is used as standard drug control. The administration of extract or standard drug (finasteride) improved the body weight of animals treated induced with BPH bringing it near the weight of normal control level.

3.2 Prostate Weight

The average weight of the prostates was 2.21 g in the animal treated with BPH control group which increased 5.39 times more compared with normal control group with weight of 0.41 g.

Therefore, BPH control group showed a significant (P<0.05) enhancement in prostate weight when compared to normal control (Table 1). The animals treated with VA extract groups using 50 and 100 mg of VA showed a decrease in prostate weight by 0.83 and 0.72 g respectively, when compared with the BPH-control group (2.21 g). Administration of VA extract or standard drug (finasteride) reduced partially the prostate weight to near normal. The animals groups treated with Finasteride served as standard drug control.

3.3 Protein Content of the Prostate

The content of protein in the rats' prostate gland was at highest (8.61 g/dl) in BPH control group and lowest (4.24 g/dl) in the normal control group. There was significant (P<0.05) increase (4.9 times rise) in protein content of the prostate in BPH-control group when compared with the value obtained for normal control (Table 1). The treatment of BPH induced groups with aqueous VA extract brought a decrease in protein content of the prostate near to normal control. Therefore, the protein content of the prostate for all the animal treated groups was statistically similar to the normal control group. Finasteride drug used as standard control.

3.4 Effect of Extract on PSA Concentration of BPH-induced Rats

Table 2 showed the plasma PSA concentration in the treated (extract and finasteride) and control groups. There was a significant (P< 0.05) enhancement of PSA concentration by 9.20 ng in the BPH control group when compared with the normal control (3.79 ng). In addition in BPH induced rats treated with 50 and 100 mg VA decreased PSA concentrations statistical similar to normal control (Table 2).

3.5 Effect of Extract on Testosterone Concentration of BPH-induced Rats

Table 2 showed that the concentration of testosterone in the BPH control group was significantly (P< 0.05) higher by 5.18 ng/mL when compared with the normal rats group which was 3.66 ng/mL. Furthermore, for the BPH induced rats, treated with the aqueous VA extract, the plasma testosterone concentrations decreased statistical (P< 0.05) near to the rats' of normal control groups. Finasteride was a drug control.

3.6 Effect of Extract on Estradiol Concentration of BPH-induced Rats

Table 2 showed the plasma estradiol concentration in the treated (extract and finasteride) and control groups. There was a significant (P< 0.05) increase of PSA concentration by 663.72 ng/ml in the BPH control group when compared with the normal control (499.27 ng/ml). Furthermore the estradiol concentration decreased significantly (P< 0.05) in the treated groups when compared with the BPH control.

3.7 Effect of Extract on Prolactin Concentration of BPH-induced Rats

Table 2 showed the plasma prolactin concentrations in the treated and control groups. In the BPH control group the concentration of prolactin was significantly higher by 7.40 ng/mL when compared to the normal control (5.77 ng/mL). The concentrations of prolactin decreased significantly (P< 0.05) in the all the treated groups when compared with the BPH control. The mean concentrations of prolactin was statistically similar (P< 0.05) when compared the normal group and each of the treated group.

3.8 Histological Examinations of the Effect VA and Finasteride in BPH-induced rats

3.8.1 Prostate of BPH-induced rats treated with 50 mg VA

Administration of the extract exhibited a decreased glandular stroma and large intra-glandular gap. The reduction was minor when compared with the BPH control group. Glandular secretions were seen with some fatty deposits in Fig. 1.

3.8.2 Prostate of BPH induced rats treated with 100 mg VA

Administration of 100 mg showed gland regeneration and are covered with flattened epithelial cells and stromal multiplication when compared to the BPH control. Shrinking and loss of tissue and deposits of fats are seen in Fig.2.

3.8.3 Prostate of BPH induced rats treated with finasteride

Finasteride group (Fig. 3) exhibited reduction in the hyperplasia of epithelial cell, showing a

diminution in epithelial cell width when compared with BPH control group. Cells reduced in size but appear normal. The administration reduced the hyperplasia of the epithelial cell, indicative of reduced epithelial layer width when compared with BPH control group in Fig. 4.

3.8.4 Prostate of BPH-induced rats without treatment

It was noted that there was an increase in the dimensions of gland, stroma and epithelial (Fig. 4) when compared with the normal control. The areas around the ducts were solidified with prominent involutions extending towards the lumen. Hyperplasia is notably seen in the stroma and glandular epithelium compared to the normal control group.

3.8.5 Prostate of rats of normal rats

The connective tissue connecting the acini and the ducts were lean and firm around the acini and ducts of the glands. The tissues were firmly

Table 1. Effect of extract of VA and finasteride body weight, prostate weight and protein content of prostate

Group	BW (g)	PW (g)	PCP (mg/tissue)
BPH + 50 mg VA	317.60±15.27^{b}	0.83±0.52^{ab}	5.76±0.61^{d}
BPH + 100 mg VA	321.60±5.68^{c}	0.72±0.36^{ab}	5.09±0.21^{bc}
BPH + Finasteride	320.40±8.99^{c}	0.63±0.23^{ab}	4.89±0.39^{b}
BPH control	270.40±8.93^{a}	2.21±0.28^{c}	8.61±0.46^{a}
Normal control	322.20±13.99^{c}	0.41±0.071^{a}	4.24±0.29^{b}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), Vernonia amygdalina (VA), body weight (BW), prostate weight (PW) and protein content of the prostate (PCP). Identical superscript (i.e. a) means there is no significant difference between the comparing group P>0.05. Non- identical superscripts (i.e. a, b, c, d) means there is significance between the comparing groups at P < 0.05.

Table 2. Effect of extract of VA and finasteride PSA, testosterone, estradiol and prolactin

Group	PSA (ng/ml)	Testosterone (ng/ml)	Estradiol (ng/ml)	Prolactin (ng/ml)
BPH + 50 mg VA	3.21±0.33^{b}	4.37±0.64^{bc}	519.53±4.86^{bc}	5.84±0.21^{a}
BPH + 100 mg VA	3.29±0.88^{bc}	4.31±0.63^{bc}	517.76±4.03^{bc}	5.84±0.17^{a}
BPH + Finasteride	2.54±0.39^{a}	3.86±0.34^{ab}	510.27±4.96^{ab}	5.79±0.55^{a}
BPH control	9.20±0.69^{e}	5.18±0.29^{d}	663.72±22.34^{d}	7.40±0.40^{b}
Normal control	3.79±0.15^{bcd}	3.66±0.56^{a}	499.27±11.06^{a}	5.77±0.10^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), Vernonia amygdalina (VA). Identical superscript (i.e. a) means there is no significant difference between the comparing group P>0.05. Non- identical superscripts (i.e. a, b, c, d, e) means there is significance between the comparing groups at P < 0.05.

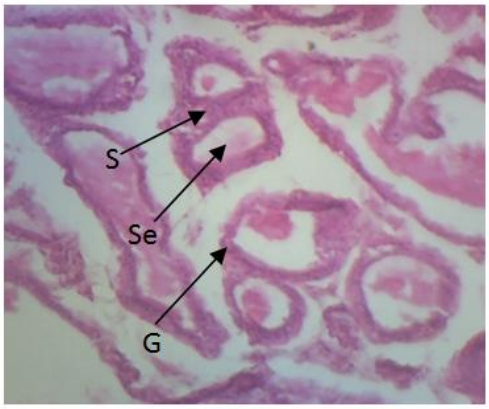

Fig. 1. Photomicrograph of prostate of rat induced with BPH and treated with 50mg VA (mag. x200). G = gland, S = stroma, Se = secretion

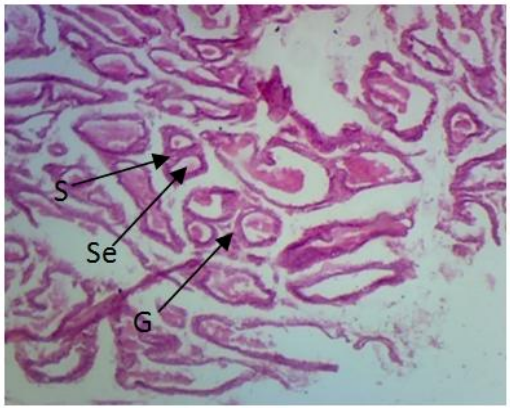

Fig. 2. Photomicrograph of prostate of rat induced with BPH and treated with 100mg VA (mag. x200). G = gland, S = stroma, Se = secretion

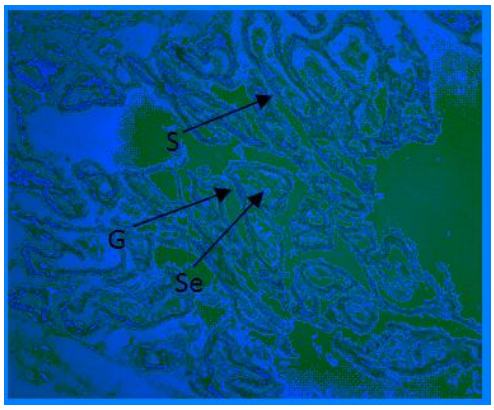

Fig. 3. Photomicrograph of prostate of rat induced with BPH and treated with Finasteride (mag. x200). G = gland, S = stroma, Se = secretion

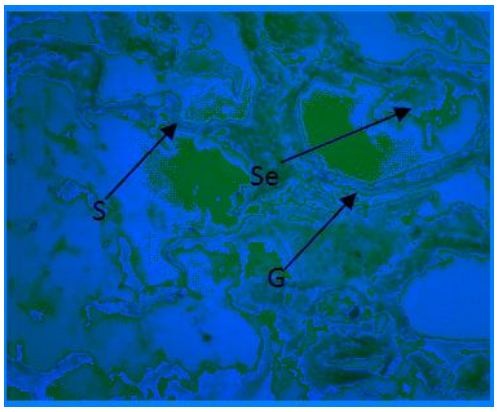

Fig. 4. Photomicrograph of prostate of rat induced with BPH and untreated (mag. x200).G = gland, S = stroma, Se = secretion

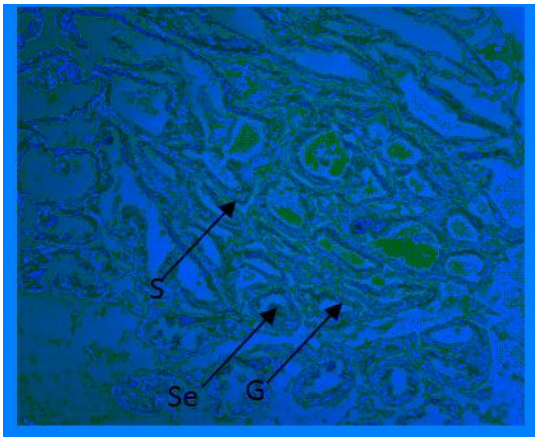

Fig. 5. Photomicrograph of prostate of rat without induction of BPH and no treatment (Normal Control). G = gland, S = stroma, Se = secretion

arranged. The epithelium was cube-shaped and normal in size in the tubules and columnar with involutions into the lumen in the oval acini. The prostate showed the fibromuscular stroma within which was embedded the glandular tissue in Fig. 5.

Histological examination of the effects of *Vernonia amygdalina* on induced prostatic hyperplasia in rats (200x magnification)

4. DISCUSSION

This study investigated the effect of administration of leaves extract of *Vernonia amygdalina* on testosterone-induced BPH. BPH is an age-related disease associated with hormonal changes, increased proliferation and suppression of apoptosis of prostatic cells [29,30]. The results obtained indicate that administration of the extract has protective effects against the development of BPH as seen in the reduction in PSA levels, improved prostate histological patterns. Prostate specific antigen is a protein produced by prostate cells. Serum PSA levels increase abnormally in patients with benign prostatic hyperplasia and prostatitis [31]. Therefore reduction of the serum PSA level can show protective effects on benign prostatic hyperplasia.

A decrease in PSA is associated with reduced prostate hyperplasia as a direct consequence of 5"-reductase inhibition or anti-inflammatory actions [32]. Though the pathogenesis of BPH remains unclear, dihydrotestosterone, a metabolite obtained from the conversion of testosterone by 5"-reductase is seen as one of

the likely causes of the derangement [33]. Consequently, inhibitors of 5"-reductase which block production of DHT ultimately slow down the development of BPH. Common inhibitors of 5"-reductase are pharmacological agents such as finasteride. However, there is strong evidence that phytochemical agents are also effective inhibitors of 5"-reductase and contributes to significant reduction in DHT concentrations [34]. It is suggested that *Vernonia amygdalina* may have 5"-reductase inhibitory activity similar to the results obtained for studies and hence prevent the development of BPH [35,36,37]. However the actual mechanism of action will need to be further investigated. Several phytochemicals have been demonstrated to reduce prostatic disorders and prostate cancer [38,39].

The variety of secondary metabolites extracted from *V. amygdalina,* explains well the diversity of the biological activities of this plant extract. Leaf extract of *V. amygdalina* was found to contain reducing sugar, polyphenolics, terpenoids, saponins, alkaloids, cardiac glycosides, steroids or triterpenes, anthraquinone and coumarins without cyanogenic glycoside [40,41,42,43,44]. Phenolic compounds identified in *V. amygdalina* can be grouped into flavonoids, tannins and caffeoyl qunic acid [45]. The histological findings showed recovery in the prostatic histoarchitecture treated with the extract proving the protective effect of *V. amygdalina* against BPH. Similar histological effects have been observed for other plants [46].

Induction of BPH led to increased of serum testosterone, estradiol and prolactin levels but treatment with *V. amygdalina* showed significant decrease in these biochemical parameters compared with BPH control group. Testosterone produced through hypothalamic-pituitary-gonadal axis activity is believed to regulate prostate growth [47]. The enzyme 5α-reductase, found in prostatic cells, catalyzes testosterone conversion into the potent androgen DHT. Dihydrotestosterone can stimulate a variety of growth factors that accelerate hyperplasia of the stromal and epithelial cells of the prostate resulting in prostatic enlargement. Inflammation can play an important role in BPH. The more the inflammation, the larger the prostate will be [48]. Experimental work has also identified age-related increases in estrogen/ estradiol levels that may increase the expression of DHT, the progenitor of BPH [49,50]. The incrimination of DHT in the pathogenesis of BPH forms the basis for the current use of 5α -reductase inhibitors in the treatment of symptomatic nodular hyperplasia [51,52].

There is a considerable number of hormones and growth factors regulating prostate growth, among them we can mention endocrine factors such as androgens (testosterone, dihydrotestosterone) [53,54], prolactin and insulin; neuroendocrine signals (5-hydroxytriptamine, noradrenalin) [55]; growth factors such as fibroblast growth factor (FGF-2), epidermal growth factor (EGF) [56]. It has been described that estrogen along with androgen stimulate prostatic stroma, upregulating androgen receptors and increasing production of the enzyme 5α-reductase which in turn increases dihydrotestosterone (DHT) in the prostate [57,58]. A nonsteroidal factor regulating prostate growth and differentiation is prolactin, which exerts its effect in an androgen independent fashion by modulating other growth factors. In men prolactin levels are increased with age and have been related to BPH development [59]. All these factors in combination promote prostate growth and proliferation.

Elevation in the number of cells in the prostate can result to a corresponding elevation in its weight. Also elevation in the number of cells in a tissue also comes with a corresponding elevation in the protein make up of the tissue [60]. In this work there was appreciable increase in protein content of the prostate which was in accordance account given by [61] but administration of the extracts significantly reduced the protein content of the prostate. Protein initiate all cell functions and pathways, identifying differentially expressed proteins between normal and pathological state, will lead to a better understanding of the cellular mechanisms involved in disease. Some proteins are down-regulated and others are up-regulated with the onset of disease, depending on a protein's specific function, undergoing disease-specific posttranslational modifications [62]. The changes of major protein fractions in patient with BPH may indicate the presence of some immunological background which may participate in the development of prostatic hyperplasia [62]. The significant elevation of serum protein may provide primitive findings to confirm the presence of BPH. *Vernonia amygdalina* administration might have caused a significant decrease of the proliferation, nucleic acids and protein synthesis in this BPH animal model.

Although the particular compound that may be responsible for the effect of the aqueous extract of bitter leaf on BPH remains unknown, we

speculate that phytoestrogens might have played a role. Phytoestrogens have been said to be beneficial in the management of BPH due to their affinity for estrogen beta receptor [63] and they are present in bitter leaf in form of lignans and flavonoids. Moreover, sesquiterpene lactones contained in bitter leaf may suppress aromatase activity thereby reducing the level of oestrogen in the body [64].

5. CONCLUSION

The results of the present investigation suggest that aqueous extract of *Vernonia amygdalina* at different dose levels inhibited prostatic hyperplasia induced by an exogenous supply of testosterone and estradiol in a rat model.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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